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Impact of adding nitrate or increasing the lipid content of two contrasting diets on blood methaemoglobin and performance of two breeds of finishing beef steers

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Short title: Blood methaemoglobin and performance of cattle

Abstract

Adding nitrate to the diet or increasing the concentration of dietary lipid are effective strategies for reducing enteric methane emissions. This study investigated their effect on health and performance of finishing beef cattle. The experiment was a two × two × three factorial design comprising two breeds (CHX, crossbred Charolais; LU, Luing); two basal diets consisting of (g/kg dry matter (DM), forage to concentrate ratios) 520:480 (Mixed) or 84:916 (Concentrate); and three treatments: (i) control with rapeseed meal as the main protein source replaced with either (ii) calcium nitrate (18 g nitrate/kg diet DM) or (iii) rapeseed cake (increasing acid hydrolysed ether extract from 25 to 48 g/kg diet DM). Steers (n = 84) were allocated to each of the six basal diet × treatments in equal numbers of each breed with feed offered *ad libitum*. Blood methaemoglobin (MetHb) concentrations (marker for nitrate poisoning) were monitored throughout the study in steers receiving nitrate. After dietary adaptation over 28 days, individual animal intake, performance and feed efficiency were recorded for a test period of 56 d. Blood MetHb concentrations were low and similar up to 14 g nitrate/kg diet DM but increased when nitrate increased to 18 g nitrate/kg diet DM (P < 0.001). An interaction between basal diet and day (P < 0.001) indicated that MetHb% was consistently greater in Concentrate- than Mixed-fed steers at 18 g nitrate/kg diet DM. Maximum individual MetHb% was 15.4% (of total Hb), which is lower than considered clinically significant (30%). MetHb concentrations for individual steers remained consistent across time. Concentrate-fed steers were more efficient (lower residual feed intake (RFI) values) than Mixed-fed steers (P < 0.01), with lower dry matter intake (DMI) (kg/d) (P < 0.001) and similar average daily gain (ADG). CHx steers were more efficient (lower RFI; P < 0.01) than LU steers with greater ADG (P < 0.01), lower DMI (/kg BW; P < 0.01) and

lower fat depth ($P < 0.001$). ADG, BW or DMI did not differ across dietary treatments ($P > 0.05$). Neither basal diet nor treatment affected carcass quality ($P > 0.05$), but CHX steers achieved a greater killing out proportion ($P < 0.001$) than LU steers. Thus, adding nitrate to the diet or increasing the level of dietary lipid through the use of cold-pressed RSC, did not adversely affect health or performance of finishing beef steers when used within the diets studied.

Keywords: beef cattle, lipid, methaemoglobin, nitrate, performance

Implications

Adding nitrate to the diet or increasing the level of dietary lipid has been shown to reduce methane from cattle. These strategies should not adversely affect animal health or performance. The use of nitrate in ruminant diets has been limited due to the potential adverse effects on health and productivity. Following four weeks adaptation, neither the addition of nitrate to the diet (18 g nitrate/kg diet dry matter) nor increased dietary lipid (48 g acid hydrolysed ether extract/kg diet dry matter) adversely affected steer health or performance. These strategies provide the potential for reducing the environmental impact of beef enterprises.

Introduction

Livestock systems, in particular ruminant production, are under increasing political pressure to reduce their greenhouse gas outputs. Breeding, enterprise and systems management and diet formulation are all possible strategies to reduce methane (CH_4) from cattle (Cottle *et al.,* 2011), with diet formulation representing one of the most practical and promising approaches. In addition to determining the

effectiveness of dietary strategies on CH_4, it is important to report their implications on health, overall performance and efficiency.

Recent interest in the controlled feeding of nitrate has been stimulated because the reduction of nitrate to ammonium in the rumen of adapted animals provides an alternative hydrogen sink to the production of CH_4 (van Zijderveld *et al*., 2010). The reduction of nitrate to nitrite and then to ammonium provides an energetically more favourable route for disposal of metabolic hydrogen produced during fermentation of feed carbohydrates in the rumen than the production of CH_4. Although nitrate has been shown in many studies to reduce CH_4 emissions from ruminants (Nolan *et al*., 2010; van Zijderveld *et al*., 2010; van Zijderveld *et al*., 2011, Hulshof *et al*., 2012; Li *et al*., 2012), the potential for its use has been hindered due to the toxicity of the intermediate product (nitrite). In the rumen, microbes rapidly reduce nitrate to nitrite and then reduce nitrite to ammonia. However, in an animal that has not been previously exposed to nitrate, the rate of reduction of nitrite to ammonia is slower than the reduction of nitrate to nitrite resulting in the accumulation of nitrite in the rumen (van Zijderveld *et al*., 2010; Jeyanathan *et al*., 2014). Absorbed nitrite binds to haemoglobin (Hb) in the blood converting it to methaemoglobin (MetHb) which is not capable of transporting oxygen to tissues. High concentrations of MetHb can cause methaemoglobinaemia, in which the functional oxygen carrying capacity of the blood is reduced. Blood MetHb is used as a marker for nitrate poisoning with a value of 30% of total Hb associated with clinical symptoms (Bruning-Fann and Kaneene, 1993). Nitrate toxicity may reduce animal performance (feed intake, growth, loss of BW), but in more severe cases may be fatal (Cockburn *et al*., 2013). Therefore, the use of nitrate in ruminant diets requires careful consideration.

Increasing the concentration of dietary lipid has been shown to reduce CH_4 emissions from ruminants (Martin *et al.,* 2010; Grainger and Beauchemin, 2011; Patra, 2013). This is achieved through various mechanisms: fatty acids are not fermented in the rumen and therefore increasing dietary lipid concentration reduces the proportion of feed which is fermentable within the rumen; lipids can also reduce CH_4 production by coating fibre particles, reducing their digestibility, and by reducing the numbers and activity of the rumen methanogens and protozoa responsible for methanogenesis (Johnson and Johnson, 1995; Patra, 2013). Dietary lipid can be increased through the addition of pure fats or oils to the diet or through the use of by-products from distilleries, breweries or plant oil extraction as ingredients in the diet (Brask *et al.,* 2013). At concentrations greater than 6% (DM basis), lipid can negatively affect feed intake and productivity, but lipid concentrations lower than 6% can be used with no adverse effects (Brask *et al.,* 2013).

When considering mitigation strategies for beef cattle, studies have been mainly focussed on breeds that are managed more intensively, with less focus on breeds suited to extensive systems. The performance characteristics of hill and upland breeds, when managed more intensively, may be considerably different to that of intensively managed breeds, although the availability of performance data is limited. For example, baseline performance data of Luing cattle, a hill and upland breed, is unavailable in the literature, even though their popularity as suckler cows is increasing in the UK and consequently the numbers of Luing calves reaching finishing units for more intensive finishing is also rising. Calf registrations of Luing and crossbred Luing calves in the UK has increased from 6165 in 2011 to 6525 in 2014 and is likely to increase further in 2015 (Agriculture and Horticulture Development Board, UK, 2015, personal communication). The large differences in

126 performance are likely a result of considerable genetic and physiological differences.
127 It is important to consider the effect of mitigation strategies across different breeds,
128 alongside their implications for health and productivity. If this differs across different
129 breeds, the industry and beef producers need to understand this if a real change in
130 CH_4 output is to be delivered in commercial practice.

131 The primary objective of the present study was to investigate the effect of
132 adding nitrate, or increasing the concentration of dietary lipid, within two contrasting
133 diets which are typical of industry practice, on the performance and carcass quality
134 of finishing beef steers of two breeds. Due to the risks associated with feeding
135 nitrate, a further objective was to investigate the effect of dietary nitrate on blood
136 MetHb concentrations.

137

138 **Materials and methods**

139 This study was conducted at the Beef and Sheep Research Centre, SRUC, UK. The
140 experiment was approved by the Animal Experiment Committee of SRUC and was
141 conducted in accordance with the requirements of the UK Animals (Scientific
142 Procedures) Act 1986.

143

144 *Experimental design, animals and diets*

145 The experiment was of a two × two × three factorial design, comprising two breeds
146 (CHX, crossbred Charolais; LU, purebred Luing), two basal diets (concentrate-straw
147 or silage-based) and three treatments selected for their potential CH_4 mitigation
148 effects (Control, Nitrate or Rapeseed Cake (RSC)). The breed types were selected
149 to represent two commercially relevant breeds where CHX cattle represent a
150 common continental sired beef breed in the UK well known for fast growth and

151 excellent carcass conformation, whilst the LU breed is typical of a more extensively
152 managed hardy hill and upland breed. The steers were fed one of two basal diets (as
153 total mixed rations) using a diet mixing wagon, consisting of (g/kg dry matter (DM))
154 forage to concentrate ratios of either 520:480 (Mixed) or 84:916 (Concentrate).
155 Within each basal diet the steers were offered one of three treatments: (i) Control
156 containing rapeseed meal as the main protein source which was replaced with either
157 (ii) Nitrate in the form of calcium nitrate (Calcinit, Yara, Oslo, Norway; 18 g nitrate/kg
158 diet DM) or (iii) an added source of lipid in the form of pelleted RSC which is a by-
159 product from cold-pressing rapeseed (acid hydrolysed ether extract (AHEE)
160 increased from 25 to 48 g AHEE/kg diet DM). The ingredient and chemical
161 composition of the experimental diets are given in Table 1. The chemical
162 composition of individual components is given in Table 2. The DM contents of
163 individual components were determined on duplicate samples twice weekly and
164 bulked feed samples (two per component) were analysed. Feed samples were
165 analysed for DM, ash, CP, ADF, NDF, AHEE, starch and neutral cellulase and
166 gammanase digestibility (Ministry of Agriculture Fisheries and Food, 1992) and gross
167 energy by adiabatic bomb calorimetry. For the Nitrate and RSC-containing diets,
168 calcium nitrate and RSC were incorporated firstly into a premix which contained the
169 concentrate portion of the diet alongside minerals and molasses. Each batch of
170 premix was mixed using a diet mixing wagon to produce a consistent premix. On a
171 daily basis each premix was then mixed with the forage portion of the diet using the
172 same mixing wagon to generate a consistent total mixed ration. Diets were mixed for
173 a minimum duration of 20 minutes.

174 In total, 84 steers (42 of each breed) were used. Thus, 14 animals (seven of
175 each breed) were allocated to each of the six basal diet × treatment combinations.

Due to the high risk of ill-health of unadapted animals gaining access to dietary nitrate, and the risks of forage-fed animals gaining access to large quantities of concentrate (e.g. acidosis), each diet × treatment combination was allocated to one pen (six pens in total). Treatments were balanced for sire within each breed, farm of origin and BW and were balanced across basal diets and treatment groups at the start of the experiment. Fresh water was provided *ad libitum* using a water trough, and diets were offered *ad libitum* to all steers using 32 electronic feeders (HOKO, Insentec, Marknesse, The Netherlands). Electronic feeders allow expression of performance in an environment close to on-farm conditions. All steers were bedded on wood fibre and sawdust to ensure that consumption of bedding did not contribute to nutrient intake.

Steers were adapted to the experimental diets in two stages. In stage one (day -56 to day -29), the animals were adapted to the basal diets. All steers were being fed the Mixed diet at the start of the adaptation period. Steers which were allocated the Concentrate diet, were adapted to the full concentrate inclusion over 4 weeks. This was undertaken at weekly intervals where diets comprising (g/kg DM) forage to concentrate ratios of 38:62, 25:75, 13:87 and 8:92 were offered during weeks 1, 2, 3 and 4, respectively. During this period, steers were trained to use the electronic feed intake recording equipment. In stage two (day –28 to day 0), steers were adapted to the treatments over a second 4 week period. Treatments (Nitrate and RSC) were progressively incorporated into the diets at 25%, 50%, 75% and 100% of the required level, on days -28, -21, -14 and -7, respectively.

Blood methaemoglobin measurements

All steers receiving dietary nitrate had blood samples taken weekly throughout the second treatment adaptation phase to monitor blood MetHb concentrations. Blood samples were taken when MetHb was expected to be greatest, i.e., 3 h after fresh feed was offered (van Zijderveld *et al.,* 2010), on the day after dietary nitrate was increased (days -27 (25%), -20 (50%), -13 (75%) and -6 (100%)) and then 15 days after maximum nitrate inclusion was achieved (day 8). To assess the long-term effects of feeding nitrate, blood samples were obtained at day 87 and day 101 (128 days after initial inclusion of nitrate). Blood samples were taken from the caudal vein into an evacuated tube (Vacuette, Griener Bio One Ltd., Gloucestershire, UK) containing heparin. MetHb concentration in blood was measured within 2 h of sampling by co-oximetry (Stat Profile Critical Care Xpress, Nova Biomedical U.K., Cheshire, UK). For each steer, dry matter intake (DMI) (kg/d) and weekly BW were assessed throughout adaptation.

56-d performance test

After full adaptation to the experimental diets, performance and feed efficiency were characterised for all steers over a 56 d test period (day 0 to 56). Steers were maintained under controlled conditions, where group sizes within the pen remained constant. Individual DMI (kg/d) was recorded for each animal using the electronic feeding equipment and BW was measured weekly using a calibrated weigh scale. Measurements of BW were obtained before fresh feed was offered. For all steers, ultrasonic fat depth was obtained at the $12^{th}/13^{th}$ rib at the start (FD0) and end (FD1) of the 56 d test using an industry-standard Aloka 500 machine (BCF technology ltd., Scotland, UK). Images were analysed using Matrox Inspector 8 software (Matrox Video and Imaging Technology Europe Ltd., Middlesex, UK). Hyslop *et al.* (2012)

assessed the consequences of alternative test lengths on the precision of average daily gain (ADG) and demonstrated that a 56-day measurement period, with weekly weighing is sufficient for characterising ADG of finishing beef cattle.

Pre-slaughter measurements and carcass quality

Steers remained within the same pens and on the same diets from the end of the 56 d test to slaughter. During this period CH_4 measurements were obtained from 76 of these steers and reported in Troy *et al*. (2015). On the day before slaughter, ultrasonic fat depth (FD2) at the $12^{th}/13^{th}$ rib was measured in all steers as described above. Steers were slaughtered in four batches of 17, 18, 21 and 25 steers on days 85, 106, 127 and 148, respectively. Steers were selected for slaughter based on BW and visual assessment of fatness. The steers were transported (approximately 1 h) to a commercial abattoir and slaughtered within 2 h of arrival. Cattle were stunned using a captive bolt, exsanguinated and subject to low voltage electrical stimulation. Following hide removal, carcasses were split in half down the mid-line and dressed to UK specification (see Meat and Livestock Commercial Services Limited beef authentication manual, www. mlcsl.co.uk, for full description). EUROP conformation and fat classifications (Fisher, 2007), based on the UK scale, were allocated to all carcasses through visual assessment using a trained assessor.

Video Image Analysis (VIA) was used to estimate EUROP classifications (conformation and fat), total lean (kg) and total fat (kg) content of the whole carcass. The VIA systems in use in the EU are automatic machines that perform carcass evaluation based on images of the half carcass. The VBS 2000 system used in this study (E+V technology GmbH, Oranienburg, Germany) has been approved by the Department for Environment, Food and Rural Affairs (Defra) for use in the UK since

2010. The system operated at the end of the slaughter line after all necessary dressing and trimming had been completed. A pneumatically operated cradle presented the left half side of each carcass for imaging. The VIA camera took two images of the half carcass, a 2-dimensional image and a pseudo 3-dimensional image using structured light (Craigie *et al.,* 2012). The VBS 2000 required information on the category of the carcass (i.e., steer) and hot carcass weight (kg) and, by combining this information with data automatically captured by the VIA system (i.e., carcass dimensions, angles, areas, colour), predicted EUROP classification and total lean and fat content of the whole carcass.

Calculations and statistical analysis

MetHb data were analysed using the mixed procedure of SAS software (SAS Institute Inc., Cary, NC, USA) using a repeated measures ANOVA including the effects of basal diet, sampling day and their interactions. Data are reported as means and standard errors of the mean (s.e.m.).

Data from three steers were unavailable as the animals were removed from the trial during the 56-d test period for health reasons unconnected to the diets and treatments imposed. Growth was modelled by linear regression of BW against test date, to obtain ADG, mid-test BW (mid-BW) and mid-test metabolic BW (mid-MBW = $BW^{0.75}$). Mean DMI over the 56 d period was expressed as kg per day or as a proportion of mid-BW and mid-MBW. Feed conversion ratio (FCR) was calculated as average DMI per day (kg/d)/ADG. Residual feed intake (RFI) was calculated as deviation of actual DMI (kg/d) from DMI predicted based on linear regression of actual DMI on ADG, mid-MBW and FD1 (Basarab *et al.,* 2003). Cold carcass weight (CCW) was calculated as a percentage of slaughter BW (SBW) to determine killing

out percentage (KO). To allow for statistical comparison, the EUROP carcass classification values were expressed on the equivalent 15 point scale (Kempster *et al.,* 1986). Statistical analyses of performance and carcass data were conducted using the mixed procedure of SAS software with the fixed effects of breed, basal diet and treatment, and the random effect of pen (and slaughter batch for carcass traits). In addition, in the analysis of FD1 and FD2 the deviation from the breed mean of FD0 was included as a covariable. The interaction effects of breed × basal diet, basal diet × treatment, breed × treatment and breed × basal diet × treatment were included in the model when these effects proved significant ($P < 0.05$). Data are reported as means with their s.e.m. Differences between means were tested using a least square means comparison test (PDIFF option of SAS). Probability values were deemed significant where $P < 0.05$ and indicated a tendency when probability values were between $P = 0.05$ and $P = 0.1$.

Results

Blood met-haemoglobin response to dietary nitrate

During the adaptation period (Table 3), blood MetHb concentrations were similar when feed contained up to 75% of total nitrate (up to -13 d) but increased when nitrate was included at the 100% level (18 g nitrate/kg diet DM) on both basal diets. During adaptation there was no difference ($P > 0.05$) in MetHb between the basal diets but blood MetHb concentrations of steers offered the Concentrate diet were consistently greater (day × basal diet interaction, $P < 0.001$) than those offered the Mixed diet from day 8 onwards.

There was a consistent individual animal response across sampling days in MetHb concentrations when animals were offered the maximum dietary nitrate

(100%, day -6 to 101). Of 28 steers, six always had MetHb concentrations less than the median MetHb for each sampling day whilst nine steers consistently had MetHb concentrations greater than the upper quartile. Figure 1 shows individual values for five steers with the smallest mean MetHb concentration and the five steers with the greatest mean concentrations and demonstrates consistency of steer response across time (from day -6 onwards, when 100% nitrate was offered). Maximum values for blood MetHb concentration (Table 3) were always less than 30% of total Hb. The greatest individual MetHb concentration value was 15.4%. There was no significant effect of breed on blood MetHb concentrations ($P > 0.05$).

56-d performance test

Neither age at the start (AgeST) nor Mid-BW differed between basal diets ($P > 0.05$; Table 4). Although not significant, the greater ADG in Mixed-fed steers (1.54 *v.* 1.41 kg/d; $P > 0.05$) was associated with greater daily DMI than Concentrate-fed steers (12.0 *v.* 11.0 kg/day; $P < 0.001$). Basal diet did not affect DMI per kg BW ($P > 0.05$). Concentrate-fed steers were more efficient (lower RFI) than Mixed-fed steers (-0.24 *v.* 0.22 kg; $P < 0.01$) due to lower daily DMI. Basal diet did not affect FD1 ($P > 0.05$).

Mid-BW, ADG, DMI and FD1 (kg/day or g/kg BW) did not differ across treatments ($P > 0.05$). An interaction between basal diet and treatment was identified for FCR and RFI ($P < 0.05$). For concentrate-fed steers, FCR did not differ between RSC and Control treatments ($P > 0.05$). There was, however, a tendency for steers offered Nitrate to have improved (lower) FCR values compared to steers offered the Control (7.40 *v.* 8.17 kg,kg; $P = 0.07$). Similarly nitrate-fed steers achieved lower RFI values than steers offered the Control treatment but this was not significant ($P >$

0.05). When offered the Mixed basal diet, neither Nitrate nor RSC treatments differed to the Control for FCR or RFI ($P > 0.05$).

To balance for BW, CHX steers were younger than LU steers at the start of test (442 *v.* 476 d; $P < 0.001$). Mid-BW did not differ between breeds ($P > 0.05$). CHX steers achieved greater ADG than LU steers (1.56 *v.* 1.39 kg/day; $P < 0.01$) with similar levels of daily DMI (11.4 *v.* 11.7 kg/day; $P > 0.05$) and lower DMI per kg BW (18.98 *v.* 19.98 g/kg BW; $P < 0.01$) to LU steers. Furthermore, FD1 was lower in CHx steers than LU steers (6.41 v. 8.28 mm; $P < 0.001$). Thus, CHx steers were more efficient than LU steers with lower FCR (7.39 *v*. 8.57 kg, kg; $P < 0.001$) and RFI (-0.2 *v.* 0.22 kg; $P < 0.01$) values.

Pre-slaughter measurements and carcass traits

Carcass traits were not affected by basal diet (Table 5), except for fat score (determined by VIA) where Concentrate-fed steers had lower fat scores than Mixed-fed steers (8.02 *v.* 9.08; $P < 0.001$). There was no difference between treatments for any carcass quality trait other than for FD2, where steers offered the RSC treatment had greater FD2 compared to the Control treatment (10.07 *v.* 8.48 mm; $P < 0.05$).

Compared to LU steers, CHX steers had lower FD2 (6.99 *v.* 10.79 mm; $P < 0.001$), greater SBW (723 *v.* 701 kg; $P = 0.051$), greater CCW (415 *v.* 369 kg; $P < 0.001$) and greater KO (57.5 *v.* 52.8%; $P < 0.001$). LU steers offered the Concentrate diet had lower CCW than those offered the Mixed diet (357 *v.* 379 kg; $P < 0.05$). For visually assigned EUROP classifications, CHX steers achieved greater conformation grades (9.90 *v.* 8.05; $P < 0.001$) and lower fat grades (9.50 *v.* 11.03; $P < 0.001$) compared to the LU steers which are in agreement with the VIA data. LU steers had greater total fat content (51.4 *v.* 40.4 kg; $P < 0.01$) and lower total meat content

(258.8 *v.* 305.7 kg; $P < 0.001$) determined by VIA than CHX steers. There were neither any treatment nor breed × treatment interaction effects for any performance or carcass-related trait ($P > 0.05$).

Discussion

Methaemoglobin response to dietary nitrate

Blood MetHb concentrations were monitored in Nitrate-fed steers for 128 d after introduction of nitrate to the diet: mean concentrations ranged from 2-7% of total Hb over this period with values in Concentrate-fed steers being consistently greater than in Mixed-fed steers. No individual MetHb measurement was greater than 15% of total Hb which was substantially less than 30% total Hb, the value associated with clinical symptoms of methaemoglobinemia (Bruning-Fann and Kaneene, 1993). This agrees with most studies in which animals were adapted slowly to dietary nitrate by increasing nitrate intakes over a period of weeks (cattle, Hulshof *et al.,* 2012, van Zijderveld *et al.,* 2011; sheep, Li *et al.,* 2012, van Zijderveld *et al.,* 2010). Slow adaptation to dietary nitrate allows the rate of reduction of nitrite to ammonia by the rumen microflora to increase and prevents accumulation of nitrite in the rumen and absorption from the rumen and thereby avoids conversion of haemoglobin to MetHb (Lee and Beauchemin, 2014). Only where nitrate was administered directly into the rumen (Takahashi *et al.,* 1998; Sar *et al.,* 2004) were greater MetHb concentrations than those found in the present study observed (34.3% and 18.37% in each of the studies, respectively), presumably because of transiently high concentrations of nitrite in the rumen generated by the method of administration. Recently, Newbold *et al.* (2014) removed steers from an experiment because MetHb concentrations in excess of 20% were observed during adaptation to nitrate. Although most steers

374 removed (8 of 9) were fed higher dietary nitrate concentrations (24 and 30 g
375 nitrate/kg diet DM) than used in the present study, one steer removed was fed 18 g
376 nitrate/kg diet DM. There was no evidence in the current experiment for adverse
377 effects of dietary nitrate over the 128 d monitoring period.

378 Measurement of blood MetHb over a 128 d period in the present study has
379 demonstrated (i) that after adaptation to nitrate, MetHb concentrations remained
380 elevated and (ii) that individual steers were consistent in their response to nitrate
381 across time, i.e., some steers always had elevated MetHb concentrations. Thus,
382 although there was no association between MetHb and animal performance in the
383 present study, in assessing the risk of methaemoglobinemia, this consistent
384 difference in response to nitrate between individual animals, together with the
385 observations of Newbold *et al.* (2014) should be noted. There was however no
386 evidence for any association between meal size and MetHb concentrations in the
387 present experiment and therefore the differences between individual animals in
388 MetHb response to nitrate are more likely to be explained by differences between
389 animals in rumen microflora, rates of absorption of nitrite from the rumen and the
390 metabolism of absorbed nitrite.

391

392 *Basal diet and treatment effects*

393 Feeding diets containing a high proportion of cereals has been shown to reduce
394 enteric CH_4 production compared to forage-based diets (Johnson and Johnson,
395 1995; Moss *et al.,* 1995; Mc Geough *et al.,* 2010; Rooke *et al.,* 2014). This strategy
396 is attractive, in that accompanying improvements in animal performance and
397 efficiency has been demonstrated (Lovett *et al.*, 2003; Mc Geough *et al.,* 2010). In
398 the present study, steers offered a higher proportion of concentrate in the diet

399 expressed better feed efficiency (RFI) than those offered a mixed forage:concentrate
400 diet. These steers were also shown to have lower levels of CH_4 production
401 compared to steers offered higher quantities of forage (Troy *et al.,* 2015).

402 Consistent results in the literature demonstrate, over the short-term, that
403 dietary nitrate can be successfully administered at levels capable of reducing CH_4
404 with no adverse effects on performance in sheep (van Zijderveld *et al.,* 2010; Li *et*
405 *al.,* 2012; El-Zaiat *et al.,* 2014), goats (Nguyen *et al.,* 2010), dairy cows (van
406 Zijderveld *et al.,* 2011) and beef cattle (Ngoc Huyen *et al.,* 2010). However, a
407 comprehensive review by Bruning-Fann and Kaneene (1993) reported a reduction in
408 feed intake when nitrate was included in the diet at 10 g nitrate/kg DM (cattle) and 30
409 g nitrate/kg DM (sheep). In agreement, Hulshof *et al.* (2012) reported a tendency for
410 calcium nitrate (fed at 22 g nitrate/kg DM) to reduce DMI by 6% in beef cattle fed a
411 sugarcane-based diet. In contrast, Sangkhom *et al.* (2012) reported improved growth
412 rates and feed conversion efficiency in growing cattle when potassium nitrate (fed at
413 36.8 g nitrate/kg DM) was included in the diet. In agreement with most studies to
414 date, feeding 18 g nitrate/kg diet DM had no adverse effects on DMI or ADG in the
415 present study. Furthermore, the Nitrate treatment tended to improve FCR of steers
416 offered the Concentrate basal diet but not the Mixed diet.

417 Van Zijderveld *et al.* (2011) reported the persistency of the effects of dietary
418 nitrate in dairy cows fed a mixed forage and concentrate diet, in which
419 measurements were obtained over four successive 24 d periods. No adverse effects
420 on milk yield or energy balance were identified, but the reductions in energy losses
421 as CH_4 were not associated with any improvements in productivity. In the present
422 study steers received the experimental diets for a minimum of 120 d and maximum
423 of 176 d. No differences in SBW, CCW, carcass grades or yields were observed

between treatments, and thus long-term feeding of nitrate did not adversely affect the level of production.

In the wider study from which this experiment is drawn, nitrate was shown to reduce CH_4 (i.e., reduced energy loss) from steers offered the Mixed diet (Troy *et al.,* 2015); however no response was observed in the present study on FCR or RFI. The benefits in reduced energy loss as CH_4 observed by Troy *et al.* (2015) may have been counter-balanced by sub-clinical effects of nitrate. In contrast, nitrate improved the FCR of steers offered the Concentrate diet, but with no aligned reduction in CH_4 (Troy *et al.,* 2015).

Although increased concentrations of dietary lipid has been shown to reduce CH_4 from ruminants (Martin *et al.*, 2010; Grainger and Beauchemin, 2011; Patra, 2013), at high concentrations in the diet lipid can negatively affect DMI and productivity (Brask *et al.*, 2013). Based on a meta-analysis, Patra (2013) demonstrated that dietary lipid concentrations in excess of 6% cause problems with productivity. Such diets with high lipid levels which negatively affect productivity are unsuitable for livestock producers due to their adverse consequences on the profitability of the enterprise. However, since the AHEE level in the RSC treatment was only 48 g/kg DM this dietary lipid concentration did not suppress DMI and no adverse effects were observed for any performance or carcass-related trait. The RSC treatment was shown to positively reduce CH_4 production by 7.5% from steers offered the Mixed basal diet but no reduction in CH_4 was observed on the Concentrate diet (Troy *et al.*, 2015).

Breed effects

448 Data on the performance and efficiency of native hill breeds in direct comparison to
449 the more common breeds of finishing cattle are sparse in the literature. The
450 performance of the LU breed on the two finishing basal diets and dietary treatments
451 considered here have not been reported to date, and thus provides novel insight into
452 the performance of this breed when managed within indoor finishing units. When
453 given diets typical of indoor finishing systems in the UK, considerable differences in
454 performance characteristics between LU and CHx steers were determined. CHx
455 steers expressed greater rates of ADG, consumed lower DMI (/day and /kg BW),
456 thus had better feed efficiency compared to the LU steers. This inefficiency will have
457 considerable impact on profitability. Although both breeds reached similar SBW, CHx
458 cattle yielded greater CCW and better EUROP classifications, both of which are
459 incorporated into current payment schemes in the UK. These differences are not
460 unexpected given the selection history of these breeds. Here LU cattle are being
461 compared with a breed intensively selected for fast growth, however in comparison
462 to the average 2014 Scottish figures for ADG of cereal-based finishing enterprises
463 (1.34 kg/d) (QMS, 2014), the Concentrate-fed LU cattle performed well (1.32 kg/d).
464 Given the animal performance results reported here, it is anticipated that the dietary
465 mitigation strategies considered will not adversely affect health or performance of
466 either breed type. Consequently, the same practical advice with regard to dietary
467 mitigation strategies can be given to commercial beef finishers looking to reduce
468 CH_4 regardless of the breed types being finished.
469

470 **Conclusions**

471 This study demonstrated that (i) the addition of nitrate to the diet or (ii) increasing the
472 level of dietary lipid through the use of cold-pressed RSC, does not adversely affect

either the performance or feed efficiency of finishing beef steers when used within either a Mixed forage/concentrate diet or a high Concentrate diet. The use of nitrate in the diet of ruminants has been limited to date due to the potential toxicity of the intermediate product (nitrite) which, at high levels, can severely impact animal health and productivity. The present study demonstrated that, following an appropriate adaptation period (four weeks), feeding of nitrate at the level considered here (18 g nitrate/kg diet DM) together with the basal diet types studied did not provide measureable adverse effects, in terms of blood MetHb response (where the maximum level reached was 15% of total Hb), animal performance and carcass characteristics. This study demonstrated that the use of RSC to increase the level of dietary lipid from 25 to 48 g AHEE/kg diet DM did not suppress DMI or ADG. Furthermore, based on the same steers Troy *et al.* (2015) demonstrated the effectiveness of these dietary treatments within a Mixed diet for reducing CH_4 (CH_4 yield was reduced by 17% and 7.5% through the use of nitrate and RSC treatments, respectively). Therefore, it is concluded that these are appropriate strategies on Mixed diets. Although the use of these mitigation strategies within a high concentrate diet was shown in the present study to provide no adverse effects on performance, they were not effective at reducing CH_4 yield (Troy *et al.,* 2015) and therefore cannot be recommended for use within high concentrate diets.

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References

Basarab JA, Price MA, Aalhus JL, Okine EK, Snelling WM and Lyle KL 2003. Residual feed intake and body composition in young growing cattle. Canadian Journal of Animal Science 83, 189–204.

Brask M, Lund P, Weisbjerg MR, Hellwing ALF, Poulsen M, Larsen MK and Hvelplund T 2013. Methane production and digestion of different physical forms of rapeseed as fat supplements in dairy cows. Journal of Dairy Science 96, 2356–2365.

Bruning-Fann CS and Kaneene JB 1993. The effects of nitrate, nitrite, and N-nitroso compounds on animal health. Veterinary and Human Toxicology 35, 237–253.

Cockburn A, Brambilla G, Fernández M-L, Arcella D, Bordajandi LR, Cottrill B, van Peteghem C and Dorne J-L 2013. Nitrite in feed: from animal health to human health. Toxicology and Applied Pharmacology 270, 209–217.

Cottle DJ, Nolan JV and Wiedemann SG 2011. Ruminant enteric methane mitigation: a review. Animal Production Science 51, 491–514.

Craigie CR, Navajas EA, Purchas RW, Maltin CA, Bunger L, Hoskin SO, Ross DW, Morris ST and Roehe R 2012. A review of the development and use of video image analysis (VIA) for beef carcass evaluation as an alternative to the current EUROP system and other subjective systems. Meat Science 92, 307-318.

El-Zaiat HM, Araujo RC, Soltan YA, Morsy AS, Louvandini H, Pires AV, Patino HO, Correa PS and Abdalla AL 2014. Encapsulated nitrate and cashew nut shell liquid on blood and rumen constituents, methane emission, and growth performance of lambs. Journal of Animal Science 92, 2214–2224.

525 Fisher, AL 2007. Beef carcass classification in the EU: an historical perspective. In:
526 Lazzaroni, C., S. Gigli, and D. Gabiña. (Eds.), Evaluation of Carcass and Meat Quality
527 in Beef and Sheep, Wageningen Academic Publishers, Wageningen, pp.19–30.
528 (EAAP publication No. 123).
529 Grainger C and Beauchemin KA 2011. Can enteric methane emissions from ruminants be
530 lowered without lowering their production? Animal Feed Science and Technology 166-
531 167, 308–320.
532 Hulshof RBA, Berndt A, Gerrits WJJ, Dijkstra J, van Zijderveld SM, Newbold JR and Perdok
533 HB 2012. Dietary nitrate supplementation reduces methane emission in beef cattle fed
534 sugarcane-based diets. Journal of Animal Science 90, 2317–2323.
535 Hyslop JJ, Duthie C-A, Ross DW, Rooke JA and Roehe R 2012. An assessment of
536 alternative test length periods when measuring liveweight change in finishing cattle
537 during feed efficiency studies. Advances in Animal Biosciences 3, 46.
538 Jeyanathan J, Martin C and Morgavi DP 2014. The use of direct-fed microbials for mitigation
539 of ruminant methane emissions: a review. Animal 8, 250–261.
540 Johnson KA and Johnson DE 1995. Methane emissions from cattle. Journal of Animal
541 Science 73, 2483–2492.
542 Kempster AJ, Cook GL and Grantley-Smith M 1986. National estimates of the body
543 composition of British cattle, sheep and pigs with special reference to trends in
544 fatness: a review. Meat Science 17, 107–138.
545 Lee C and Beauchemin KA 2014. A review of feeding supplementary nitrate to ruminant
546 animals: nitrate toxicity, methane emissions, and production performance. Canadian
547 Journal of Animal Science 94, 557-570.
548 Li L, Davis J, Nolan J and Hegarty R 2012. An initial investigation on rumen fermentation
549 pattern and methane emission of sheep offered diets containing urea or nitrate as the
550 nitrogen source. Animal Production Science 52, 653–658.

551 Lovett D, Lovell S, Stack L, Callan J, Finlay M, Conolly J and O'Mara FP 2003. Effect of
552 forage/concentrate ratio and dietary coconut oil level on methane output and
553 performance of finishing beef heifers. Livestock Production Science 84, 135–146.
554 Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to
555 the farm scale. Animal 4, 351–365.
556 Mc Geough EJ, O'Kiely P, Hart KJ, Moloney AP, Boland TM and Kenny DA 2010. Methane
557 emissions, feed intake, performance, digestibility, and rumen fermentation of finishing
558 beef cattle offered whole-crop wheat silages differing in grain content. Journal of
559 Animal Science 88, 2703–2716.
560 Ministry of Agriculture Fisheries and Food 1992. Analysis of Agricultural Materials., 2nd
561 Edition . Her Majesty's Stationary Office., London.
562 Moss AR, Givens DI and Garnsworthy PC 1995. The effect of supplementing grass silage
563 with barley on digestibility, in sacco degradability, rumen fermentation and methane
564 production in sheep at two levels of intake. Animal Feed Science and Technology 55,
565 9–33.
566 Newbold JR, van Zijderveld SM, Hulshof RBA, Fokkink WB, Leng RA, Terencio R, Powers
567 WJ, van Adrichem PSJ, Paton ND and Perdok HB 2014. The effect of incremental
568 levels of dietary nitrate on methane emissions in Holstein steers and performance in
569 Nelore bulls. Journal of Animal Science 92, 5032–5040.
570 Ngoc Huyen LTN, Do HQ, Preston TR and Leng RA 2010. Nitrate as fermentable nitrogen
571 supplement to reduce rumen methane production.T. R. Preston, R. S. Sansoucy, J. S.
572 Correa, and H. Oorio, editors. Livestock Research for Rural Development 22, 146.
573 Nguyen NA, Khuc TH, Duong NK and Preston TR 2010. Effect of calcium nitrate as NPN
574 source on growth performance and methane emissions of goats fed sugar cane
575 supplemented with cassava foliage. In: T. R. Preston and B. Ogle, editors. Mekarn
576 conference on livestock production, climate change and resource depletion. Pakes,
577 Laos. p. 8.

578 Nolan JV, Hegarty RS, Hegarty J, Godwin IR and Woodgate R 2010. Effects of dietary
579 nitrate on fermentation, methane production and digesta kinetics in sheep. Animal
580 Production Science 50, 801–806.
581 Patra AK 2013. The effect of dietary fats on methane emissions, and its other effects on
582 digestibility, rumen fermentation and lactation performance in cattle: A meta-analysis.
583 Livestock Science 155, 244–254.
584 Quality Meat Scotland (QMS) 2014. Cattle and Sheep Enterprise Profitability in Scotland.
585 QMS, Edinburgh, UK.
586 Rooke JA, Wallace RJ, Duthie C-A, McKain N, de Souza SM, Hyslop JJ, Ross DW,
587 Waterhouse T and Roehe R 2014. Hydrogen and methane emissions from beef cattle
588 and their rumen microbial community vary with diet, time after feeding and genotype.
589 British Journal of Nutrition 112, 398–407.
590 Sangkhom I, Preston TR, Khang DN and Leng RA 2012. Effect of potassium nitrate and
591 urea as fermentable nitrogen sources on growth performance and methane emissions
592 in local “Yellow” cattle fed lime (Ca(OH)2) treated rice straw supplemented with fresh
593 cassava foliage. Livestock Research for Rural Development 24, 27.
594 Sar C, Santoso B, Mwenya B, Gamo Y, Kobayashi T, Morikawa R, Kimura K, Mizukoshi H
595 and Takahashi J. 2004. Manipulation of rumen methanogenesis by the combination of
596 nitrate with β1-4 galacto-oligosaccharides or nisin in sheep. Animal Feed Science and
597 Technology 115, 129–142.
598 Takahashi J, Ikeda M, Matsuoka S and Fujita H 1998. Prophylactic effect of L-cysteine to
599 acute and subclinical nitrate toxicity in sheep. Animal Feed Science and Technology
600 74, 273–280.
601 Thomas C 2004. Feed into Milk: an Advisory Manual. Nottingham: Nottingham University
602 Press.
603 Troy S, Duthie C-A, Hyslop JJ, Roehe R, Ross DW, Wallace RJ, Waterhouse A and Rooke
604 JA 2015. Effectiveness of nitrate addition and increased oil content as methane

mitigation strategies for beef cattle fed two contrasting basal diets. Journal of Animal Science 93, 1-9.

Van Zijderveld SM, Gerrits WJJ, Apajalahti JA, Newbold JR, Dijkstra J, Leng RA and Perdok HB 2010. Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. Journal of Dairy Science 93, 5856–5866.

Van Zijderveld SM, Gerrits WJJ, Dijkstra J, Newbold JR, Hulshof RBA and Perdok HB 2011. Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. Journal of Dairy Science 94, 4028–4038.

631 **Table 1** *Ingredient composition and calculated chemical composition of experimental diets*

Basal Diet	Mixed			Concentrate		
Treatment	Control	Nitrate	RSC	Control	Nitrate	RSC
Ingredient composition, g/kg DM[1]						
Grass silage	189	193	192			
Whole crop barley silage	331	334	334			
Barley straw				84	84	83
Barley	328	374	287	740	797	700
Rapeseed meal	123	45	16	145	63	19
Rapeseed cake			142			167
Calcinit[2]		24			24	
Molasses	19	21	20	21	21	21
Minerals[3]	9	10	9	10	10	10
Chemical composition, g/kg DM[4]						
Dry matter (g/kg)	543	539	541	863	860	865
CP	143	148	145	133	138	136
ADF	252	240	253	145	130	143
NDF	376	361	367	237	220	223
Starch	234	257	211	430	458	408
AHEE	23.9	23.4	44.1	27.0	26.6	51.0
Ash	48	44	50	36	31	37
ME (MJ/kg DM)	11.6	11.4	12.1	12.0	11.9	12.7
GE (MJ /kg DM)	17.7	17.2	18.1	18.1	17.7	18.7

632 [1]Ingredient composition is the mean of the daily diets received by the animals across the
633 experimental period.
634 [2]Contained (g/kg DM): nitrate, 769; Ca, 229.
635 [3]Contained (mg/kg): Fe, 6036; Mn, 2200; Zn, 2600; Iodine, 200; Co, 90; Cu, 2500; Se 30;
636 (µg/kg): vitamin E, 2000; vitamin B12, 1000; vitamin A, 151515; vitamin D, 2500
637 [4]Chemical composition is the mean of 2 analyses per treatment, apart from DM which is the
638 mean of 32 analyses.
639 RSC, Rapeseed Cake; AHEE, acid hydrolysed ether extract; ME, metabolisable energy; GE,
640 gross energy
641
642

643 **Table 2** *Chemical composition of feeding stuffs (g/kg DM*)

	Grass Silage	WCBS	Straw	Barley	RSM	RSC	Molasses
DM (g/kg)	273	557	807	867	896	901	971
CP	150	103	16	104	367	318	67
NDF	486	575	826	163	326	209	0
ADF	345	390	551	86	243	197	0
Starch	5.7	122	0	571	52	41	0
AHEE	36	12.6	14	30	27	170	0
Ash	80	41	63	22	79	75	147
NCGD (% DM)			44	88	73	78	0
ME (MJ /kg DM)	11.4	10.75	6.5	13.05	10.9	15.15	12.7
GE (MJ /kg DM)	19.1	16.0	15.0	18.7	19.3	22.4	14.25
pH	4.1	5.3					

644 WCBS, whole crop barley silage; RSM, rapeseed meal; RSC, rapeseed cake; DM, dry
645 matter; AHEE, acid hydrolysed ether extract; NCGD, neutral cellulase and gammanase
646 digestibility; ME, metabolisable energy; GE, gross energy
647 ME values (Thomas, 2004), were either estimated from near infra red spectroscopy (silage
648 and WCBS), from NCDG and AHEE (barley, RSM and RSC) or from tabulated values for
649 feed composition (straw and molasses).
650

Table 3 *Changes in mean and maximum individual blood MetHb concentration (% total Hb) in relation to nitrate intake and long-term nitrate feeding*

Day[1]	-27	-20	-13	-6	8	87	101		Significance		
Nitrate (%)[2]	25	50	75	100	100	100	100	SEM	Day	Diet	Day*Diet
Mixed	0.26^{a}	0.78^{ab}	0.80^{ab}	3.50^{c}	2.16^{bc}	1.29^{ab}	3.60^{c}	0.61	***	*	***
Concentrate	0.32^{a}	0.62^{a}	0.98^{a}	2.80^{b}	4.53^{bc}	6.46^{d}	4.61^{c}				
Maximum	0.60	2.00	3.20	9.50	11.60	15.40	10.30				

Number of steers = 28

[1]Day relative to start of 56 day performance period.

[2]Nitrate as percentage of maximum level of intake (100% = 18 g/kg DM).

Within a row, means without a common superscript differ ($P < 0.05$).

*P < 0.05; ***P <0.001.

Table 4 *Effect of breed (B), basal diet (D) and treatment (T) on growth, feed intake and feed efficiency of Charolais-sired (CHX) and purebred Luing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Rapeseed cake (RSC)*

Basal Diet	Mixed						Concentrate									
Treatment	Control		RSC		Nitrate		Control		RSC		Nitrate		Significance[1]			
Breed	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU	SEM	B	D	T
AgeST (days)	445	478	434	474	437	474	449	481	444	482	441	465	7.9	***	ns	ns
Mid-BW (kg)	611	601	591	594	605	596	594	567	588	573	602	571	22.2	ns	ns	ns
Mid-MBW (kg)	123	121	120	120	122	121	120	116	119	117	121	117	3.4	ns	ns	ns
ADG (kg/day)	1.56	1.48	1.71	1.42	1.61	1.46	1.47	1.32	1.46	1.19	1.53	1.44	0.092	**	ns	ns
DMI (kg/day)	11.4	12.8	11.7	11.8	12.1	12.2	11.1	11.2	11.1	10.9	10.7	11.0	0.50	ns	***	ns
DMI/BW(g/kg)	18.7	21.2	19.8	19.9	19.9	20.5	18.8	19.8	18.8	19.0	17.8	19.1	0.49	**	ns	ns
DMI/MBW(g/kg)	93.0	105.0	97.7	98.0	98.7	101.3	92.5	96.7	92.7	92.8	88.0	93.5	2.52	**	ns	ns
FCR (kg, kg)[2]	7.45	8.69	6.86	8.39	7.61	8.49	7.59	8.85	7.70	9.33	7.16	7.67	0.421	****	ns	ns
RFI (kg)[3]	-0.27	0.76	-0.15	-0.06	0.44	0.62	-0.27	0.12	-0.22	-0.10	-0.71	-0.18	0.228	**	**	ns
FD1 (mm)[4]	6.31	8.83	6.87	9.12	5.89	7.53	6.85	8.34	6.65	8.49	5.87	7.25	0.650	***	ns	ns

Number of animals = 81; AgeST, Age at start of test; Mid-BW, mid-test BW; Mid-MBW, mid-test metabolic BW; ADG, average daily gain at the end of the 56 d test; FCR, feed conversion ratio; RFI, residual feed intake; FD1, fat depth at the 12/13th rib at the end of the 56 d test

[1]Breed × Diet and Breed × Treatment interaction effects were not significant for all variables ($P > 0.05$)

[2]Diet × Treatment interaction ($P < 0.05$): Concentrate-Nitrate different to Concentrate-RSC ($P < 0.05$); Concentrate-Control different to Concentrate-Nitrate ($P = 0.07$)

[3]Diet × Treatment interaction ($P < 0.05$): Mixed-Nitrate different to Mixed-RSC ($P < 0.01$)

[4]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

*P < 0.05; **P <0.01; ***P <0.001.

Table 5 *Effect of breed (B), basal diet (D) and treatment (T) on carcass traits of Charolais-sired (CHX) and purebred Luing (LU) steers fed either a Mixed- or Concentrate-based diet containing one of three treatments: Control, Nitrate or Rapeseed cake (RSC)*

Basal Diet	Mixed						Concentrate									
Treatment	Control		RSC		Nitrate		Control		RSC		Nitrate		Significance[1]			
Breed	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU	CHX	LU	SEM	B	D	T
FD2 (mm)[2]	6.31	10.17	7.84	12.90	6.47	9.28	6.60	10.83	8.03	11.50	6.67	10.06	0.907	***	ns	*
CCW (kg)[3]	430	370	406	385	408	382	417	365	411	361	418	346	9.4	***	ns	ns
KO (%)	58.4	52.3	56.7	53.7	57.0	53.1	58.4	52.8	57.3	53.0	57.4	51.5	0.88	***	ns	ns
SBW (kg)	738	710	717	719	717	720	713	694	718	682	729	672	19.5	ns	ns	ns
CONF	10.3	8.0	9.7	8.3	9.7	8.3	10.0	8.0	9.4	8.0	10.3	7.7	0.34	***	ns	ns
FAT	10.0	10.6	10.0	12.0	8.7	10.6	9.4	10.7	9.4	11.0	9.4	11.3	0.44	***	ns	ns
CONF (VIA)	10.7	8.0	9.8	8.0	9.6	7.6	10.3	7.4	9.9	6.9	9.8	6.7	0.53	***	ns	ns
FAT (VIA)	7.9	10.7	8.3	10.2	7.5	10.0	7.6	8.7	7.6	9.3	6.6	8.7	0.47	***	***	ns
TOTFat (kg)	46.4	51.3	42.1	70.7	38.1	50.1	41.8	44.5	34.7	45.9	37.8	42.8	5.95	**	ns	ns
TOTMeat (kg)	314.0	256.6	294.2	261.9	299.0	270.0	308.9	260.7	306.3	259.2	312.0	244.5	8.09	***	ns	ns

Number of animals = 81; FD2, pre-slaughter fat depth at the 12/13th rib; CCW, cold carcass weight; KO, killing out %; SBW, slaughter BW; CONF, EUROP conformation (15 pt scale) assigned by visual assessor; FAT, EUROP fatness (15pt scale) assigned by visual assessor; CONF (VIA), conformation grade (15pt scale) assigned by VIA; FAT (VIA), fatness grade (15pt scale) assigned by VIA; TOTFat; total fat content predicted by VIA; TOTMeat, total meat content predicted by VIA.

[1]Breed × Treatment and Basal Diet × Treatment interaction effects were not significant for all variables ($P > 0.05$).

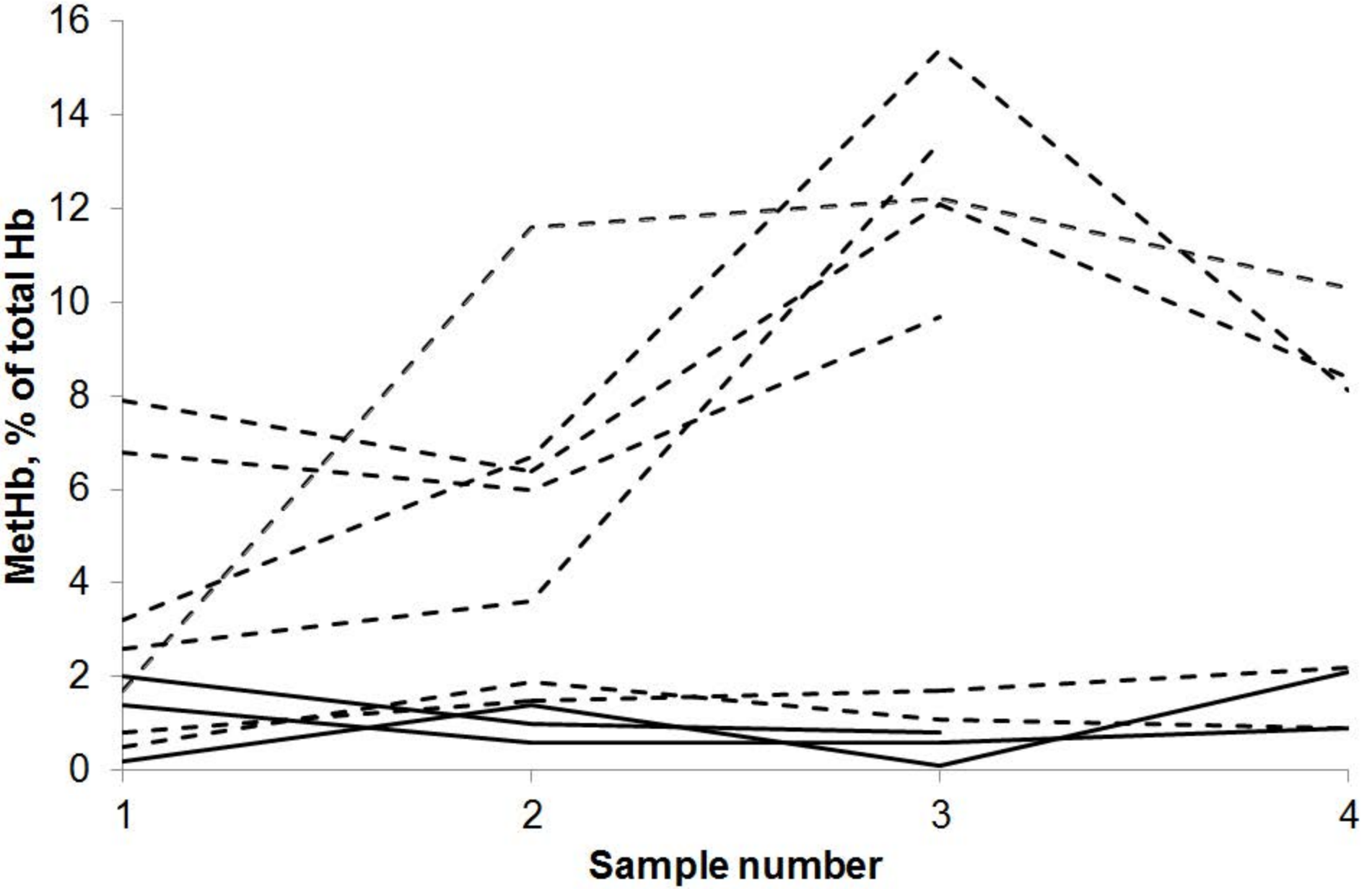
[2]Deviation from breed mean of FD0 (measured at start of 56-d performance test) fitted as covariable

[3]Breed × Diet interaction ($P < 0.05$): CHX-Concentrate different from LU-Concentrate and LU-Mixed ($P < 0.001$); CHX-Mixed different from LU-Concentrate and LU-Mixed ($P < 0.001$); LU-Mixed different from LU-Concentrate ($P < 0.01$). *P < 0.05; **P <0.01; ***P <0.001.

690 **List of Figure Captions**

691 **Figure 1** Changes in Met-haemoglobin (MetHb) concentrations (% total blood Hb) when fed
692 100% dietary nitrate (18 g nitrate/kg DM) for 5 steers with overall smallest and overall
693 greatest mean MetHb concentrations. Solid lines and dashed lines represent the Mixed and
694 Concentrate basal diets, respectively. Samples 1 to 4 refer to sampling days -6, 8, 87 and
695 101, respectively. Each line represents an individual animal. Sample 4 was not present for 3
696 animals as had been already been sent for slaughter before day 101.

697


16
14
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MetHb, % of total Hb
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Sample number